T449A Shaker-IR background with different hydrophobicities according to the Wimley-White hydrophobicity scale (Ala, Cys, or Trp) and charges (Glu, Asp, Arg, Lys) and measured the inactivation kinetics. Mutant channels were transiently expressed in tsA_201 cells. All ionic current experiments were performed with excised inside-out patches. The introduction of an ionizable cysteine and the small but neutral alanine dramatically slowed entry into the inactivated state (the inactivation time constants for Shaker mutant T449A/I470C, T449A/I470A and T449A were 1.4 s, 250 ms and 50 ms, respectively). We could not detect any current on mutant T449A/I470X (X = Asp, Glu, Trp) but the transfection with a mixture of mutant and wild-type expression plasmids expressed currents. These heterotetramers had slower inactivation kinetics with respect to the T449A channels. Almost all of the channels had a midpoint voltage for activation in the range −50 mV to −20 mV. The only exception was the I470C mutant which had a large shift in voltage dependence (midpoint ~5 mV). Mutant channels containing Arg or Lys (I470R, I470K) did not give functional expression either as homotetramers or as heterotetramers. Our results show that not the size but the physicochemical properties of the side chains (hydrophobicity and charge) at position 470 determine the inactivation kinetics of voltage-gated K^+ channels, which may reflect interaction of the side chains with permeant ions.

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Voltage Sensor Domain Mutations Involved in the Kv1.2 Channel Activation via MD Simulations

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Voltage-gated cation channels (VGC) are membrane-bound proteins responsible for the generation and propagation of the action potential that regulates a number of critical biological events such as skeletal muscle contraction, neuron activity and hormone secretion. These channels are equipped with voltage sensor domains (VS) that act as electrical devices highly sensitive to transmembrane (TM) voltage variations () that modulate the gating state of VGC. Despite major structural conservation within VS from the VGC family, there are marked sequence variations among them related to their diverse kinetic rates for *up-down* turnover. For instance, the VS kinetics is markedly distinct between Kv and Nav channels, a feature that complies with their respective role in the fast and slow phases of the action potential. Currently, original results concerning single point mutations have identified key amino acids directly affecting the kinetic rates of Shaker-like channels activation, such as I237A (Lacroix J.J. *et al*, 2012), F290A (Schwaiger *et al*, 2013), and more recently I287T/V363T (Lacroix J.J. *et al*, 2013).
Here, by benefiting from the well understood activated and resting states of Kv1.2 we investigate the molecular nature of such mutations affecting the *up-down* VS turnover. Specifically, we use all atom MD simulations to investigate the structural effects of the referred mutations to the sensing domain. Anticipating our results, such amino acid changes provoke minor structural rearrangements in the *up* and *down* states of the VS, thus indicating that these mutations could actually be affecting the intermediate states withing the activation pathway of the channel.

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Atomistic Modeling of Ion Conduction through Voltage-Sensing Domains

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Voltage-sensing domains (VSDs) are modular membrane protein units that sense changes in the membrane electrostatic potential, and through conformational changes, regulate a specific function. The VSDs of voltage-dependent K^+, Na^+ and Ca^{2+} channels do not conduct ions under physiological conditions, but they can become ion-permeable under pathological conditions through mutations in the voltage sensor, particularly of S4 basic side chains [1,2]. Relatively little is known about the underlying mechanisms of conduction through VSDs. The most detailed studies have been performed on Shaker K^+ channel variants that include the mutation of the outermost Arg residue in S4 to a smaller, uncharged side chain[1,2]. Ion conduction through the Shaker VSD is manifested in electrophysiology experiments as a separate voltage-dependent inward current that appears when the VSDs are in their resting state conformation[1]. Only monovalent cations permeate the Shaker VSD through a narrow and twisted pathway after reaching a vestibular region on the extracellular side of the VSD[1,2]. This permeation pathway is, at least in part, the same as that one followed by the S4 basic side chains during voltage-dependent activation. We sampled VSD ion conduction events on the microsecond timescale under a membrane potential using experimentally validated models of the Shaker VSD[3] in a resting state in order to elucidate the molecular mechanisms of ion conduction, gating, and selectivity. This work was supported by NIH Grants F30CA171717 to M.L.W. R01GM098973 to FT, P01GM86685 to DJT SHW, and P41GM103712-S1 to the National Resource for Biomedical Supercomputing.

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Cys Mutation + MTS Caution is Needed in Interpretation of Arg Reaction in the S4 Transmembrane Segment of a Voltage Sensing Domain (VSD) of a Voltage Gated Channel: Results of Quantum Calculations

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In all previous work on voltage gated channels, mutation of the arginines on the S4 segment of the VSD, then reaction with an MTS reagent, followed by channel shutdown, has been taken to mean that the arginine was exposed on the surface from which the reagent was applied. This may require more care in interpretation. Cysteine is smaller than the arginine by about the size of the reactive sulfonate on the MTS; The mutation leaves a large cavity where the arginine side chain had been, so the MTS can reach the cysteine, possibly via the omega pore. The backbone atoms need not move. The distance between S4 and S2 or S3 remains largely unchanged. Salt bridges, (e.g., R297-E183) however, are disturbed; when cysteine is in the reactive (negative) form, it constitutes a charge reversal mutation, as the arginine was (presumably) positive. Quantum calculations on configurations of this region for R300C of the VSD of Kv1.2 show that the cys anion can fold away from the cavity where it could react, in a manner dependent on the water and protons present. See the preprint posted at http://arxiv.org/abs/1309.1373. Optimizations have been done at BLYP/6-31G** level. Acknowledgement: Computations were done at the Brookhaven National Laboratory CFN cluster, and the CUNY hpc facility.

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Quantum Calculations Show How the Water at the Gate of the Voltage Gated Kv1.2 Channel Plays a Major Role in Determining Conduction through the Gate

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We have carried out quantum calculations to optimize the structure of the gate region of a Kv1.2 (with hydrogens added as in the pdb 3Lut structure) channel as well as the structure below the gate as far as the T1 segment, starting from the X-ray structure. A key result: A PVPV–>PVVV in silico mutant disrupts the water structure. The normal gate opens just enough to allow a hydrated K^+ to enter the cavity that separates the gate from the selectivity filter. The mutation also alters the electrostatic potential. In addition, we have calculated the result of protonation of the H418 below the gate, which shows approximately a 3 Å rotation toward the pore on protonation, enough to alter the potential at the gate; deletion of H418 is reported to produce a non-functional channel(1). With Cl- ion at the gate, but not K^+, a water structure forms in the optimized channel structure, and appears to be fairly stable; this may already select against anions. Calculations in cases that involve K^+ include bond order and charges on all atoms, enabling calculation of electrical potentials. Optimizations were done initially at HF/6-31G* level; most have been confirmed at BLYP/6-31G** level, with little change in any case tested. Acknowledgement: Computations were done at the Brookhaven National Lab CFN cluster and the CUNY hpc facility.

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Turning a Small into Large Conductance K-Channel - How Far Can We Go?

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Potassium channels are membrane proteins that allow the passage of K^+ ions across the hydrophobic core of the membrane. They display an extremely conserved signature sequence that elicits high ion transport rates and exquisite discrimination between ions with similar radii. Despite of this conservation,